REMARKS

The Office Action dated May 19, 2005 has been carefully reviewed and the foregoing remarks are made in response thereto. In view of the following remarks and amendments to claims 1, 2, 19, 25-30 and 34, Applicants respectfully request reconsideration and reexamination of this application and the timely allowance of the pending claims.

Claims 1, 19, 25-30 and 34 have been amended. No prohibited new matter has been added. Specifically, claims 1, 19 and 34 have been amended to spell out the abbreviation "LDL" for low-density lipoprotein. Support for this amendment can be found throughout the specification. Support for the amendment to claims 25-30 may be found in original claims 15-18 and Figures 5 and 6. Claim 1 has also been amended to claim "recombinant" apolipoprotein. Support for this amendment may be found in paragraphs [0072] and [0097] to [0101]. Claim 34 has been amended to recite the limitation of claim 2.

The word "days" was inadvertently omitted from the phrase "The LDL suspension is stable for at least 7 days and can be stored at -20°C under argon or nitrogen." The amendment to the specification corrects this omission.

I. Summary of the Office Action.

1. Claims 1-41 are currently pending.

2. The Examiner objects to the phrase "LDL suspension is stable for at least 7..." that appears on page 25, paragraph 104 of the specification as omitting a time frame for stability.

3. The Examiner has objected to the use of the abbreviation "LDL" in the claims.

4. Claims 1-33 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite for reciting the phrase "solid lipid core."

5. The Examiner has rejected claims 1-17, 22-29 and 34-41 under 35 U.S.C. § 102(b) as purportedly anticipated by Versluis *et al.*

6. The Examiner has rejected claims 28 and 29 under 35 U.S.C. § 102(b) as purportedly anticipated by U.S. Patent No. 6,245,753 to Byun *et al.*

7. The Examiner has rejected claims 1, 5-9, 11-17, 25, 27, 39 and 41 under 35 U.S.C. § 102(b) as purportedly anticipated by Westesen *et al.*

8. The Examiner objects to claims 18-21 and 30-33, because they are dependent on rejected independent claims, but would be allowable if rewritten in independent form.

9. No claims are allowed.

II. Interview Summary

Applicant and Applicant's representative sincerely thank the Examiner for the courtesy of an interview. Applicant's representative, Einar Stole, and the Examiner discussed all pending claims and all rejections of the pending claims. Specifically, the parties discussed the disclosure of Versluis *et al.* Versluis *et al.* specifically disclose the use of liposomes, which contain a phospholipid ***bilayer***, not the monolayer of an LDL particle, to overcome the problems of particle stability associated with the use of LDL particles for drug delivery. Also, neither Byun *et al.* or Westesen *et al.* teach cholesterol conjugates. Based on these inadequacies of the cited references, Applicants respectfully submit that the cited references neither disclose or suggest the claimed invention and request withdrawal of the outstanding rejections.

III. Response to the Office Action.

1. Objection to the Specification.

The Examiner has objected to the phrase "LDL suspension is stable for at least 7..." that appears on page 25, paragraph 104 of the specification as omitting a time frame for stability. Applicants have amended the final sentence of paragraph [00104] on page 25 to correct this omission. Accordingly, the Applicants respectfully request withdrawal of this objection.

2. Objection to the Claims.

The Examiner has objected to the use of the abbreviation "LDL" in the claims. Claims 1, 19 and 33 have been amended to spell out the abbreviation "LDL" for low-density lipoprotein. Accordingly, Applicants respectfully request withdrawal of this objection.

3. Claim rejections: Indefiniteness.

Claims 1-33 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite for reciting the phrase "solid lipid core." Specifically, the Examiner alleges that

"'solid' must be more definitely defined in the claims, since it is not certain whether 'solid' has high viscosity like gelatin or it is solid as wood." Applicants respectfully traverse this rejection.

The test for definiteness under 35 U.S.C. § 112, second paragraph, is whether "those skilled in the art would understand what is claimed when the claim is read in light of the specification." *Orthokinetics, Inc. v. Safety Travel Chairs, Inc.*, 806 F.2d 1565, 1576, 1 USPQ2d 1081, 1088 (Fed. Cir. 1986). In reviewing a claim for compliance with 35 U.S.C. 112, second paragraph, the examiner must consider the claim as a whole to determine whether the claim apprises one of ordinary skill in the art of its scope and, therefore, serves the notice function required by 35 U.S.C. 112, second paragraph, by providing clear warning to others as to what constitutes infringement of the patent. *See, e.g., Solomon v. Kimberly-Clark Corp.*, 216 F.3d 1372, 1379, 55 USPQ2d 1279, 1283 (Fed. Cir. 2000). *See also Metabolite Labs., Inc. v. Lab. Corp. of Am. Holdings,* 370 F.3d 1354, 1366, 71 USPQ2d 1081, 1089 (Fed. Cir. 2004) ("The requirement to 'distinctly' claim means that the claim must have a meaning discernible to one of ordinary skill in the art when construed according to correct principles. Only when a claim remains insolubly ambiguous without a discernible meaning after all reasonable attempts at construction must a court declare it indefinite.").

The Examiner has not applied this test. The Examiner alleges that the phrase a "solid lipid core" is indefinite because "solid" may mean that the solid core has a "high viscosity like gelatin or it is solid as wood." The Examiner provides no review of the specification or analysis to support her conclusion that a skilled artisan would not understand the claims in light of the specification. In contrast, the specification fully describes the claimed invention such that a skilled artisan could discern its meaning. The Examiner appears to prefer further definition, but has not met her burden and has not demonstrated that the claims are "insolubly ambiguous without discernible meaning." *Id.*

Even a claim term that is not used or defined in the specification is not necessarily indefinite if the meaning of the claim term is discernible. *Bancorp Services, L.L.C. v. Hartford Life Ins. Co.*, 359 F.3d 1367, 1372, 69 USPQ2d 1996, 1999-2000 (Fed. Cir. 2004). A fundamental principle of 35 U.S.C. 112, second paragraph, is that applicants are their own lexicographers. Claim terms may be essentially in whatever terms they choose so long as any special meaning assigned to a term is clearly set forth in the specification. See MPEP § 2111.01.

As noted by the court in *In re Swinehart*, 439 F.2d 210, 160 USPQ 226 (CCPA 1971), a claim may not be rejected solely because of the type of language used to define the subject matter for which patent protection is sought.

> In this case, however, the term "solid core" is specifically defined.
>
> As used herein, the term "solid core" means that portion of an artificial LDL particle enclosed by a spherical phospholipid monolayer. The solid core may comprise one or more lipids, including but not limited to triacylglycerols, cholesterol, cholesterol esters, fatty-acyl esters, and the like.

This express definition clearly identifies the meaning of the term "solid core" in a manner that satisfies the requirements of 35 U.S.C. 112, second paragraph. Accordingly, Applicants respectfully request withdrawal of the rejection of claims 1-33 as indefinite.

4. Claim rejections: Versluis *et al.*

The Examiner has rejected claims 1-17, 22-29 and 34-41 under 35 U.S.C. § 102(b) as purportedly anticipated by Versluis *et al.* The Examiner equates the liposomes of Versluis *et al.* with the claimed artificial LDL particles. Applicants respectfully traverse this rejection.

Versluis *et al.* specifically disclose a liposome for the delivery of a conjugated lipophilic daunorubicin prodrug. Versluis *et al.* specifically describe the design and selection of a liposome to overcome problems associated with low-density lipoproteins (LDL). Specifically, Versluis *et al.* cite the limited availability of LDL and the fact that "the incorporation of cytotoxic drugs into native LDL often induces altered physiological behavior of the particles" and "causes subtle changes in the structure of apoB, which provoke in vivo uptake by mechanisms other than the LDL receptor (references omitted)." Versluis *et al.* at page 1; column 2. Versluis *et al.* explain that they "recently developed small (29 nm) liposomes" to overcome the problems associated with LDL. Versluis *et al.* at page 2; column 1; first paragraph. Furthermore, in the "Experimental Procedures" section, Versluis *et al.* characterize the "LAD-Containing (apoE) Liposomes" (page 2; column 2; last paragraph) as comprising a "phospholipid bilayer" (see page 3; column 1; first paragraph).

From inspecting Versluis *et al.*, as a whole, it is clear that the reference discloses specific liposomes, "synthetic LDL-like particles" (page 2, column 1; paragraph 1), and not the claimed

artificial LDL particles comprising a phospholipid ***monolayer***. Liposomes and LDL particles are different structures with different properties. Most notably, liposomes contain phospholipid bilayers, whereas LDL contain a phospholipid monolayer. In a liposome bilayer, the phosphate head groups of the phospholipids are oriented to both the inside and outside of the liposome. In contrast, the phosphate head groups are oriented to the outside of the particle, whereas the fatty acyl chains are oriented toward the center of the LDL.

The Examiner has erred in conflating the term liposome with particles that do not include a phospholipid bilayer. The skilled artisan understands a liposome to comprise a lipid bilayer, and Versluis *et al.* specifically designed and constructed a liposome, including a phospholipid bilayer (see Versluis *et al.* at page 3; column 1; paragraph 1), to overcome the recited problems associated with LDL particles. There is no ambiguity that the compounds of Versluis *et al.* contain a bilayer. It is equally unambiguous that the term "liposome" specifically refers to a structure containing a bilayer. For example, the Oxford Dictionary of Biochemistry and Molecular Biology defines liposomes, in relevant part, as "any small, roughly spherical, artificial vesicle consisting of a continuous bilayer or multibilayer of complex lipids enclosing some of the suspending medium." (See Oxford Dictionary of Biochemistry and Molecular Biology, revised Edition (2000)). A brief survey of the World Wide Web confirms this usage. For example, the International Liposome Society, an organization dedicated to the promotion of the science and technology related to the assembly of lipids and amphiphilic molecules into particulate or vesicular structures (liposomes, etc.) and their medical and non-medical uses, refers to liposomes synonymously with bilayers. (See attached "Liposome Evolution"). The Society identifies 1965 as the year when closed lipid bilayer vesicles were first described, and 1967 as the year "liposome" was introduced "to describe closed lipid bilayer vesicles." (*Id.*). An article in Drug Delivery Technology entitled "Liposomal Formulations" also equates liposomes with phospholipid bilayers: "It was reported more than 40 years ago that when phospholipids are subject to aqueous environments, closed bilayer structures (liposomes) ..."

Versluis *et al.* specifically teach liposomes containing a daunorubicin prodrug. Liposomes were expressly selected to avoid problems associated with LDL. Versluis *et al.* do not disclose the artificial LDL particles of claims 1-17, 22-29 and 34-41, and the Examiner has no basis for equating liposomes with particles comprising a phospholipid ***monolayer***.

Liposomes, and specifically the liposomes disclosed by Versluis *et al.* (see page 3; column 1), by definition and usage in the art refer to "closed lipid bilayer vesicles." Accordingly, Applicants respectfully request the withdrawal of the rejection of claims 1-17, 22-29 and 34-41 as allegedly anticipated by Versluis *et al.*

5. Claim rejections: Byun *et al.*

The Examiner has rejected claims 28 and 29 under 35 U.S.C. § 102(b) as purportedly anticipated by U.S. Patent No. 6,245,753 to Byun *et al.* The Examiner alleges that the heparin-cholesterol derivatives of Byun *et al.* anticipate the conjugates of claims 28 and 29. Applicants respectfully traverse this rejection.

Claims 25-30 are drawn to cholesterol conjugates of adriamycin and tetracycline. Byun *et al.* neither teaches nor suggests conjugates of adriamycin and tetracycline. Accordingly, Applicants respectfully request the withdrawal of the rejection of claims 28 and 29 as allegedly anticipated by Byun *et al.*

6. Claim rejections: Westesen *et al.*

The Examiner has rejected claims 1, 5-9, 11-17, 25, 27, 39 and 41 under 35 U.S.C. § 102(b) as purportedly anticipated by Westesen *et al.* The Examiner equates the native LDL particles disclosed in Westesen *et al.* with the claimed artificial LDL particles. Applicants respectfully traverse this rejection.

Claims 1 and 34 have been amended to recite "at least one ***recombinant*** apolipoprotein." Westesen *et al.* study and characterize native and drug-loaded human LDL using NMR techniques to determine the physiochemical characteristics of drug-loaded LDL. Westesen *et al.* do not teach or suggest artificial LDL particles containing a recombinant apolipoprotein. Accordingly, Applicants respectfully request withdrawal of the rejection of claims 1, 5-9, 11-17, 25, 27, 39 and 41 as allegedly anticipated by Westesen *et al.*

III. Conclusion.

Applicants believe that the above-referenced application is in condition for allowance. Reconsideration and withdrawal of the outstanding rejections and early notice of allowance to that effect is respectfully requested.

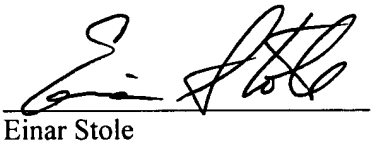
EXCEPT for issue fees payable under 37 C.F.R. § 1.18, the Director is hereby authorized by this paper to charge any additional fees during the entire pendency of this application, including fees due under 37 C.F.R. §§ 1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account No. 13-3250, reference No. 02012.4121. This paragraph is intended to be a **CONSTRUCTIVE PETITION FOR EXTENSION OF TIME** in accordance with 37 C.F.R. § 1.136(a)(3).

If the Examiner finds that a telephone conference would further prosecution of this application, the Examiner is invited to contact the undersigned at 202-835-7553.

Respectfully submitted,

MILBANK, TWEED, HADLEY & McCLOY LLP

Date: November 21, 2005

By: 

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lipophilin *another name for* myelinproteolipid. *See* **myelin protein**.

lipophorin any member of the major class of lipid-transporting proteins found in the hemolymph of insects. In locusts the apoprotein is synthesized in the fat body as an 85 kDa polypeptide. *See also* **apolipophorin**.

lipophosphoglycans *see* **GPI anchor**.

lipopolysaccharide *abbr.*: LPS; any of a group of related, 10 kDa, structurally complex components of the outer leaflet of the outer membrane of Gram-negative bacteria. Lipopolysaccharide molecules consist of three covalently linked regions: lipid A, core oligosaccharide, and an O side chain. The innermost layer, lipid A, which is responsible for the toxicity of the lipopolysaccharide, consists of six fatty acyl chains (sometimes hydroxylated) linked in various ways to two glucosamine residues. The branched core oligosaccharide contains ten saccharide residues, several of them unusual, and has a structure that appears to be similar in closely related bacterial strains. The outermost O side chain, which is highly variable and determines the antigenic specificity of the organism, is made up of many (≈50) repeating units of a branched tetrasaccharide containing further unusual sugar residues.

lipopolysaccharide *N*-acetylglucosaminyltransferase EC 2.4.1.56; an enzyme of the lipopolysaccharide core biosynthetic pathway in enteric bacteria; it catalyses a reaction between UDP-*N*-acetyl-D-glucosamine and lipopolysaccharide to form UDP and *N*-acetyl-D-glucosaminyllipopolysaccharide. Example from *Salmonella typhimurium*: database code RFAK_SALTY, 381 amino acids (43.10 kDa). There is little similarity between the given example and the corresponding enzyme in *Escherichia coli*. *See also* **glycolipid**.

lipoprotein any conjugated, water-soluble protein in which the nonprotein moiety consists of a lipid or lipids. The lipid may be triacylglycerol, cholesterol, or phospholipid, or a combination of these. *Compare* **proteolipid**.

lipoprotein(a) one or two molecules of apolipoprotein(a) linked by a disulfide bridge to **apolipoprotein B**-100; it may be a physiological inhibitor of plasminogen activation. Levels in human plasma (which range from undetectable to 100 mg dL^{-3}) show correlation with the incidence of atherosclerosis.

lipoprotein lipase EC 3.1.1.34; *abbr.*: LPL; *other names*: clearing factor lipase; diglyceride lipase; diacylglycerol lipase. An enzyme that catalyses the hydrolysis of triacylglycerol to diacylglycerol and fatty-acid anion. Its function, after uptake of dietary lipid, is to hydrolyse triacylglycerols in chylomicrons and very-low-density lipoproteins, to diacylglycerols and fatty-acid anions; the fatty acids are then taken into peripheral tissues for futher metabolism; it will also hydrolyse diacylglycerols. It acts in the presence of apolipoprotein C-II on the luminal surface of vascular endothelium. It is a **GPI-anchor** dimer, and is released by heparin, etc. Example (precursor) human: database code LIPL_HUMAN, 475 amino acids (53.10 kDa).

lipoprotein signal peptidase *see* **prepilin-leader peptidase**.

liposome **1** a natural lipid globule suspended in the cytoplasm of some cells. **2** any small, roughly spherical, artificial vesicle consisting of a continuous bilayer or multibilayer of complex lipids enclosing some of the suspending medium. Liposomes are formed by allowing complex lipids to 'swell' in aqueous solution sometimes with the aid of sonication. They are used experimentally as models of biological membranes and therapeutically for entrapment of drugs, enzymes, or other agents with a view to their more effective delivery to target cells. Originally, such a structure was termed a cellule (when bilayered), smectic mesophase, spherule, or spherulite. *See also* **liquid crystal**.

lipotaurine the 7,13-dihydroxy-2-*trans*-octadecenoyl amide of taurine. It is found in the lipid fraction of *Tetrahymena thermophila*.

lipoteichoic acid any of a group of **teichoic acids** that contain lipid, are present in the membranes of all species of Gram-positive bacteria so far examined, and, unlike wall teichoic acids, are extractable with hot water or phenol.

lipotropic *or* **lipotrophic** tending to prevent the accumulation of, or to remove, abnormal amounts of lipid (in a tissue or organ, e.g. adipose tissue, liver).

lipotropic agent *or* **lipotropic substance** any substance capable of preventing or correcting fatty infiltration of the liver caused by choline deficiency.

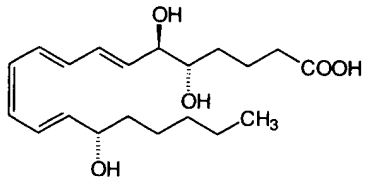
lipotropic hormone *or* **lipotrophic hormone** *a former name for* **lipotropin**.

lipotropin *or* **lipotropic hormone** (*abbr.*: LPH) either of two polypeptides, β- and γ-lipotropin, that can be isolated from the anterior and intermediary lobes of mammalian pituitary glands and are characterized by their ability to elicit lipolysis in adipose tissue. β-Lipotropin corresponds in sequence to the 91 C-terminal amino-acid residues of **proopiomelanocortin**, and γ-lipotropin corresponds to the 58 N-terminal residues of β-lipotropin. In fact, they are of no greater lipotropic potency than corticotropin and α-melanotropin, and whether they have any intrinsic physiological function is still uncertain. Both β- and γ-lipotropins are found in normal human blood. *Other names*: lipolytic hormone; adipokinetic hormone; *former names*: lipotrophin; lipotrophic hormone.

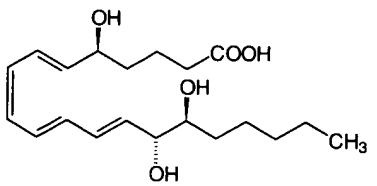
lipotropism the state or quality of being lipotropic.

lipovitellin a ≈135 kDa phospholipoprotein that, together with phosvitin, constitutes the bulk of the yolk proteins of the eggs of oviparous vertebrates. These two proteins are synthesized and secreted by the liver as a single large molecule, **vitellogenin**, which is taken up by the ovary and split into the two components.

lipoxin *abbr.*: LX; any of a group of eicosanoids containing a conjugated tetraene structure and having three hydroxyl groups. They are generated by the action of **lipoxygenase** enzymes on polyunsaturated fatty acids (arachidonate and eicosapentaenoate). The major lipoxins, A_4 and B_4, are derived from arachidonate; lipoxin A_4 (LXA_4) is (7*E*,9*E*,11*Z*,13*E*)-(5*S*,6*R*,15*S*)-5,6,15-trihydroxy-eicosa-7,9,11,13-tetraen-1-oate; lipoxin B_4 (LXB_4) is (6*E*,8*Z*,10*E*,12*E*)-(5*S*,14*R*,15*S*)-5,14,15-trihydroxy-eicosa-6,8,10,12-tetraen-1-oate. LXA_4 has been found to cause contraction of guinea-pig lung strips, but not ileum; LXA_4 and LXB_4 cause dilatation of arterioles.



lipoxin A_4



lipoxin B_4

lipoxygenase **1** EC 1.13.11.12; *systematic name*: linoleate:oxygen 13-oxidoreductase; *other names*: lipoxidase; carotene oxidase. An enzyme that catalyses the oxidation by dioxygen of linoleate to (9*Z*,11*E*)-(13*S*)-13-hydroperoxyoctadeca-9,11-dienoate (*abbr.*: 13(*S*)-HpODE); iron is a cofactor. **2** any member of a group of dioxygenase enzymes that catalyse the oxidation of polyunsaturated fatty acids to a particular corresponding hydroperoxide. Such enzymes are

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LIPOSOMAL FORMULATIONS

Articles: Issues Associated with Large-Scale Production of Liposomal Formulations

By: Tony Nakhla, PhD; Mike Marek, MS, RPh; & Tom Kovalcik, PhD

INTRODUCTION

It was reported more than 40 years ago that when phospholipids are subjec aqueous environments, closed bilayer structures (liposomes) spontaneous that can encapsulate part of the aqueous medium in their interior.[1]

Initially, liposomes were attractive to biophysicists as model systems for bi membranes. The lipid bilayer structures of liposomes mimic the barrier pro of biomembranes, and therefore, they offered the potential of examining the behavior of membranes of known composition. Thus, by altering the lipid composition of the bilayer or the material incorporated, it was possible to e differences in membrane properties. Model membranes have been used extensively to study lipid-protein interactions, in addition to membrane fun and structural properties.[2-6]

Liposomes can be formulated and processed to differ in size, composition, and lamellarity and accordingly, a wide range of compounds may be incorp into either the lipid or trapped aqueous space. Such flexibility has presente several potential applications to scientific investigators to which liposomes since been adapted.[7-10]

The biodegradable and non-toxic nature of phospholipid vesicles proposes these formulations are amenable to administration without serious side effe a result, liposomes are frequently used as drug delivery vehicles. Further, liposomes are regarded as suitable carriers because they can serve as a de system for the sustained release of an associated compound.

One of the basic goals of chemical therapeutics is to deliver the drug efficie and specifically to the site of disease. Some drugs may be delivered in thei form whereas others require a carrier in order to reach and enter their final destination because a) they are rapidly cleared from the area of introductio circulation or b) they are obstructed by biological barriers, which they cann permeate. Liposomes can alter the biodistribution of entrapped substances protect the enclosed materials from inactivation by the host defense mechanisms.[11] Therefore, liposomes can be used as vehicles to achieve sp delivery of therapeutic drugs to target organs. In addition, liposomes can re toxicity of antimicrobial[12], antiviral[13], and chemotherapeutic[7-9,14] agents, an have demonstrated the ability to modulate or potentiate the immunogenicity antigenic substances, that is, function as immunological adjuvants.[15,16] Accordingly, there has been a myriad of drugs and antigens incorporated i liposomes to achieve those objectives. More recently, liposomes have beer

demonstrated to be efficient vehicles for gene therapy.[10, 17, 18]

SIZE REDUCTION

With the many potential uses presented by these model membranes, the therape applications of liposomes are dependent on the physical integrity and stability of t bilayer structure. Lipid-based formulations can be devised as site-specific drug de vehicles that a) are readily cleared by the Kupffer cells of the liver and the macrophages[19,20] or b) evade detection of the active substance by the reticuloen system (RES) and efficiently deliver liposome-incorporated material to target tissu organ or tumor.[21,22]

There are numerous techniques for liposome preparation, and the resulting vesic be large, small, and of unilamellar or multilamellar nature.[23] Multilamellar vesicles composed of numerous concentric bilayers, are produced from mechanical agitat dispersion of dried lipid with an aqueous phase. Mechanical agitation is the simpl method for production of MLV that produces a suspension of large liposomes tha very heterogeneous in size and exhibit a relatively low level of aqueous encapsul addition, these heterogenous liposome formulations manifest relatively short circu half-lives in mammals, are cleared rapidly from sites of administration, and are di within the RES. However, homogenous liposome formulations that exhibit reduce vesicle diameters are advantageous with respect to extended circulation half-life, consequently, enhanced uptake by tissues and organs. These parameters are cr the in vivo behavior of liposomal drug delivery systems. Large unilamellar vesicle can be prepared from MLV to exhibit the characteristics that are beneficial for enl delivery of the incorporated material. The most common method for LUV prepara extrusion of MLV under pressure through membranes of known pore sizes.[24] The LUVs are utilized to optimize the incorporation of a desired compound within lipos to limit the permeability of the membrane to the entrapped material, and to alter h circulation in attempts to enhance the therapeutic efficiency of a liposomal formul Accordingly, size distribution is a critical parameter with respect to the pharmacol and pharmacodynamic behavior of biologically active substances that are site-sp targeted in vivo.

STABILIZATION OF LIPOSOMES

The stability of liposomes should meet the same standards as conventional pharmaceuticals. It is imperative that the chemical and physical stability of the ve question are maintained. Chemical stability involves prevention of both the hydro ester bonds in the phospholipid bilayer and the oxidation of unsaturated sites in tl chain. Chemical instability can lead to physical instability or leakage of encapsula from the bilayer and fusion and aggregation of vesicles. Approaches that can be increase liposome stability involve efficient formulation and lyophilization. Formul involves the selection of the appropriate lipid composition and concentration of th bilayer, in addition to the aqueous phase ingredients, such as buffers, antioxidant chelators, and cryoprotectants. Charge-inducing lipids, such as phosphatidylglyce be incorporated into the liposome bilayer to decrease fusion, while cholesterol an sphingomyelin can be included in formulations in order to decrease permeability leakage of encapsulated drugs. Buffers at neutral pH can decrease hydrolysis. If buffer is necessary during processing, for example, in active loading of drug, the buffer should be readjusted to neutral pH or exchanged by ultrafiltration. Addition antioxidant, such as sodium ascorbate can decrease oxidation. Oxygen potential kept to a minimum during processing by nitrogen purging solutions whereas ethylendiamine tetraacetic acid (EDTA) can be added as a metal chelator to decr free radical damage. High temperature and excessive shear that may be encount during processing can be avoided by sizing lipids with an extrusion device rather high-pressure homogenization. Freeze-dried liposome formulations should includ lyoprotectant, preferably a non-reducing disaccharide, such as trehalose and suc which have been shown to stabilize liposomes during freezing and

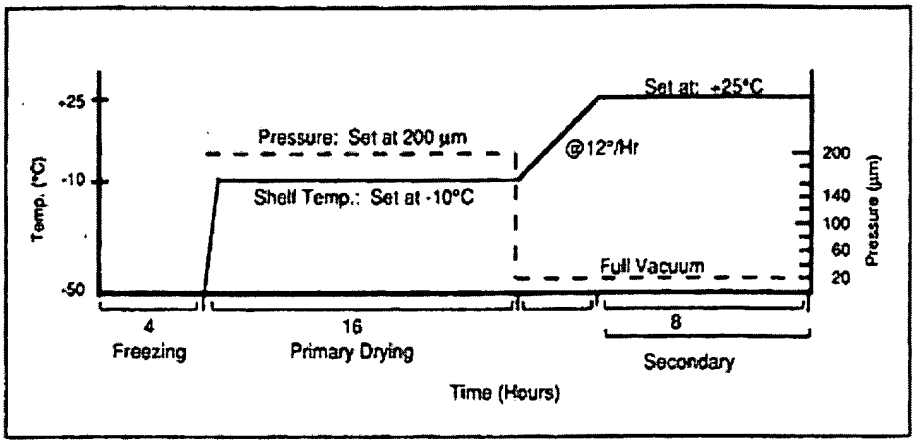
dehydration/rehydration. In general, successful formulation of stable liposome dr products includes the following[25]:

1. Processing with fresh, purified lipids and solvents.
2. Avoidance of high temperatures and excessive shear force.
3. Maintenance of low oxygen potential (nitrogen purging).
4. Use of antioxidants and/or metal chelators.
5. Formulating at neutral pH.
6. Use of a lyoprotectant when freeze-drying.

FREEZE-DRYING OF LIPOSOMES

Freeze-drying involves freezing of the product then removal of water at low temp and high vacuum via sublimation of ice. This part of the freeze-drying cycle is ref as primary drying. Adsorbed water is further removed at above freezing temperat low vacuum during secondary drying, resulting in the freeze-dried product. In ger moisture levels of freeze-dried products are designed to be less than 3%. A typic freeze-drying cycle is shown in Figure 1.[26] Liposome bilayer membranes may be damaged during the freeze-drying cycle both by mechanical stress caused by hig pressures vesicle membranes are exposed to during ice crystal formation and ch from increased concentrations of solute during freezing and dehydration. Cryopro have been shown to decrease vesicle fusion and leakage caused by both freeze- and the freeze-drying process. Cryoprotectants are noneutectic in nature, that is not crystallize, forming an amorphous frozen matrix upon cooling. The freeze pro generally occurs very quickly in the presence of cryoprotectants upon cooling to t freezing point depression. This effect is known as supercooling. Supercooling de the vesicle exposure to high concentrations of solute caused by slow ice crystalli and results in a more uniform frozen matrix. Solutes that act as cryoprotectants t undergo a transition upon freezing from a viscous gel to a hard glass with less m mobility. This is referred to as the glass transition temperature (*Tg'*). Glass transit temperatures of various cryoprotectants are listed in Table 1.[27] Critical to succes freeze-drying is that the product temperature remain below Tg' during primary dr avoid "shrinkage" or product collapse. Disaccharides have collapse temperatures between –30°C and -35°C. The use of cryoprotectants with high collapse temper allows for faster rates of freeze-drying at higher product temperatures. The use o non-reducing disaccharide sugars sucrose and trehaloses have been shown effe decreasing physical damage of liposomes during freezing and freeze-drying. This protective effect appears to be sugar specific as monosaccharides glucose and f have less effect.[28,29] Disaccharides appear to interact directly with the phospholi membrane, possibly via hydrogen bonding. Cryoprotection of liposomes is greate formulating with these sugars at high concentration (5% to 20%). Vesicle fusion c decreased at lower concentrations than are needed to minimize leakage. Preven leakage requires the sugar be present both inside and outside the liposome. Adv of formulating liposomes with trehalose include less reactive than reducing sugar *Tg'* than sucrose, high melting temperature (100°C at 2% moisture), low hygrosc and FDA approval as an injectable ingredient.[30]

FIGURE 1



A freeze-drying cycle.

Table 1 Collapse Temperatures During Lyophilization for Various Solutes

Compound	Collapse temperature (°C)
Monosaccarides	
Glucose	-40
Fructose	-48
Sugar Alcohols	
Sorbitol	-45
Inositol	-27
Disaccharides	
Sucrose	-32
Lactose	-32
Maltose	-32
Trisaccharides	
Raffinose	-26
Oligosaccharides	
Cycloinulohexaose	-25
Polysaccharides	
Ficoll	-20
Dextran	-9
Polymers	
Polyvinylpyrrolidone	-23
Polyethyleneglycol	-13

CHALLENGES

Making liposome formulations exhibiting narrow size distribution has been a form challenge under the demanding conditions and large volumes required for pharmaceutical production. At moderate volumes, size reduction is often not cons and unpredictable membrane clogging occurs. To manufacture lipid-based drug d systems on a commercial scale, the extrusion process must work with 10 to 100,

volumes.

As a result of the encouraging data that has been generated by several laborator liposome therapy has moved into the clinic. Accordingly, an efficient, robust syste can reproducibly generate homogenous unilamellar liposome formulations that e: diameters in the 100- to 200-nm range and limit the amount of lost material is a n for the pharmaceutical, biotechnology, and cosmetic industries. In addition, stabil sterility, and processing are critical issues in the development of large volume lip formulations under cGMP conditions.

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BIOGRAPHIES

Dr. Tony Nakhla is a Senior Scientist at Cardinal Health Pharmaceutical Tech-n Services and has served in his current capacity since January 2000. Dr. Nakhla's responsibilities include the development and modification, in accordance with cG cGMP, of novel drug formulations. Further, lyophilization cycles are developed ar optimized. The formulations in question are foremost, although not limited, to the lipid-based drug delivery systems. Previous industrial experience has focused on study of novel forms of w-3 polyunsaturated fatty acids as infant formula and nutr supplements, in addition to serving as the Director of Product Development for Li Biomembranes, Inc. Dr. Nakhla earned his PhD from the Department of Biochem Memorial University.

Mike Marek is a Senior Scientist at Cardinal Health Pharmaceutical Technologie Services. He has extensive experience in parenteral product develop-ment and C scale-up of protein and peptide-based pharmaceuticals and in the development c nuclear medicines. He is a graduate of the University of Illinois College of Pharm the University of New Mexico College of Pharmacy.

Dr. Thomas R. Kovalcik is a graduate of Duquesne University (BS Pharmacy) a University of Iowa (PhD Pharmaceutics). His 14 years of industrial experience inc ophthalmic product design and managing product development. He oversees enc testing, analytical method transfer, R&D project management, stability testing, an formulations at Cardinal Health Pharmaceutical Technologies & Services in Albu New Mexico.

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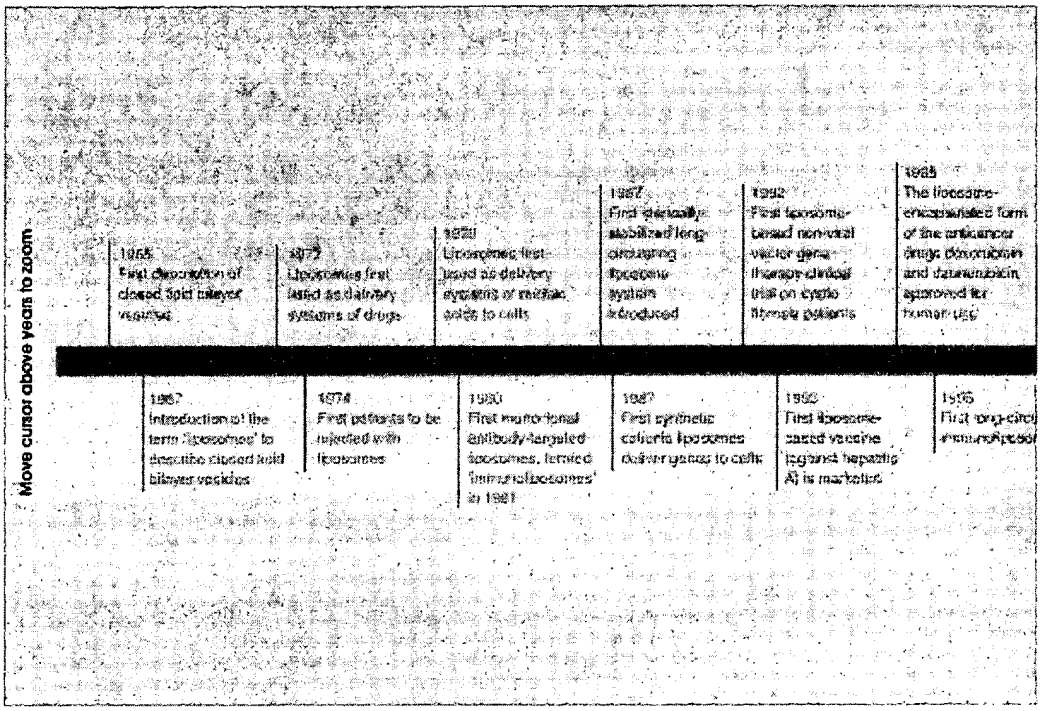
Liposome Evolution

Lipid bilayers have been known for decades, often described and referred to as black lipid films, Liposo
of closed, lipid bilayer vesicles were described for the first time in the seminal paper by Bangham, Sta
Watkins in 1965. Since then liposome technologies have rapidly evolved to systems employed in a wi
applications. Below are the most important milestones in the process of liposome evolution:



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